Scientific Opinion on an application by Bayer CropScience and Monsanto (EFSA-GMO-NL-2009-75) for placing on the market of genetically modified glufosinate-ammonium- and glyphosate-tolerant oilseed rape MS8 3 RF3 3 GT73 and subcombinations, which have not been authorised previously (i.e. MS8 3 GT73 and RF3 3 GT73) independently of their origin, for food and feed uses, import and processing, with the exception of isolated seed protein for food, under Regulation (EC) No 1829/2003

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Scientific Opinion on an application by Bayer CropScience and Monsanto (EFSA-GMO-NL-2009-75) for placing on the market of genetically modified glufosinate-ammonium- and glyphosate-tolerant oilseed rape MS8 × RF3 × GT73 and subcombinations, which have not been authorised previously (i.e. MS8 × GT73 and RF3 × GT73) independently of their origin, for food and feed uses, import and processing, with the exception of isolated seed protein for food, under Regulation (EC) No 1829/2003

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

The EFSA Panel on Genetically Modified Organisms (GMO) previously assessed the three single events that are combined to produce the three-event stack oilseed rape (OSR) MS8 × RF3 × GT73. In this Scientific Opinion, the GMO Panel assessed the three-event stack OSR and subcombinations that have not been authorised previously (i.e. MS8 × GT73 and RF3 × GT73), independently of their origin, for food and feed uses, import and processing, with the exception of isolated seed protein for food. The combination of OSR events, MS8, RF3 and GT73, in the three-event stack OSR does not raise issues relating to molecular, agronomic/phenotypic or compositional characteristics requiring further investigations. In line with previous assessments and considering the scope of this application, the GMO Panel did not find indications of safety concern for food and feed with trace levels of glyphosate oxidoreductase (GOX)v247 protein derived from the three-event stack OSR; whereas, the GMO Panel cannot assess the safety of three-event stack OSR products rich in protein, such as rapeseed protein isolates in feed. As the risk assessment of the three-event stack OSR could not be completed for products rich in protein, such as rapeseed protein isolates, the GMO Panel is not in a position to complete the food and feed safety assessment of subcombinations within the scope of this application (i.e. MS8 × GT73 and RF3 × GT73). The two-event stack OSR MS8 × RF3 is outside the scope of this application. Considering the scope of this application, the mode of action of the introduced traits and the data available for the three-event and two-event stack OSR MS8 × RF3, the GMO Panel considered that different combinations of the events, MS8, RF3 and GT73, would not raise environmental concerns.

Keywords: GMO, oilseed rape (*Brassica napus*), MS8 × RF3 × GT73, herbicide tolerance, PAT, CP4 EPSPS, GOXv247, Regulation (EC) No 1829/2003

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2009-00890

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Summary

Following the submission of application EFSA-GMO-NL-2009-75 under Regulation (EC) No 1829/2003[1] from Bayer CropScience AG and Monsanto (referred to hereafter as the applicant), the European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms (hereafter referred as GMO Panel) was asked to deliver a Scientific Opinion on the safety of genetically modified glufosinate-ammonium- and glyphosate-tolerant oilseed rape MS8 × RF3 × GT73 (hereafter referred as 'three-event stack oilseed rape') and specific subcombinations[2] (hereafter referred as 'subcombinations independently of their origin' according to the Commission Implementing Regulation (EU) No 503/2013[3]). The scope of application EFSA-GMO-NL-2009-75 is for the placing on the market of oilseed rape MS8 × RF3 × GT73 and subcombinations that have not been authorised previously (i.e. MS8 × GT73 and RF3 × GT73), independently of their origin, for food and feed uses, import and processing, with the exception of isolated seed protein for food.

The term 'subcombination' refers to any combination of two of the events present in the three-event stack oilseed rape. Subcombinations occur as segregating progeny in the harvested seeds of oilseed rape MS8 × RF3 × GT73 (embryo and albumen), and their safety is evaluated within the assessment of the three-event stack oilseed rape in Section 4 of the present EFSA GMO Panel Scientific Opinion.

'Subcombination' also covers combinations of two of the events MS8, RF3 or GT73 that have either been or could be produced by conventional crossing through targeted breeding approaches. These are oilseed rape stacks that can be bred, produced and marketed independently of the three-event stack. These stacks, excluding oilseed rape MS8 × RF3 that is not in the scope of this application, are risk assessed in Section 5 of the present EFSA GMO Panel Scientific Opinion.

In accordance with the EFSA GMO Panel Guidance Document applicable to this application (EFSA, 2007), 'where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability, (b) expression of the events and (c) potential interactions between the events'. For application EFSA-GMO-NL-2009-75, previous assessments of the three single events (MS8, RF3 and GT73) and the two-event stack oilseed rape MS8 × RF3 provided a basis to evaluate the three-event stack oilseed rape and its subcombinations.

In delivering its Scientific Opinion, the GMO Panel considered the data available on the three-event stack oilseed rape and subcombinations, the scientific comments submitted by the Member States and the relevant scientific literature.

The three-event stack oilseed rape was produced by conventional crossing to combine three single oilseed rape events. The parental oilseed rape lines MS8 (expressing Barnase and phosphinothricin-acetyl-transferase (PAT) proteins) and RF3 (expressing Barstar and PAT proteins) have been developed for the production of hybrid seeds MS8 × RF3 resulting in higher yield due to hybrid vigour. oilseed rape MS8, RF3 and MS8 × RF3 were previously assessed all together in respective EFSA GMO Panel Scientific Opinions, and no concerns on their safety were identified. oilseed rape GT73 (expressing glyphosate oxidoreductase (GOX)v247 and CP4 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) proteins) was previously assessed by the GMO Panel, and no safety concerns on its safety were identified, except for materials rich in protein, such as isolated seed protein, for which an assessment could not be completed. No safety issue was identified by the updated bioinformatic analyses, nor reported by the applicant concerning the three single oilseed rape events, since the publication of the previous EFSA GMO Panel Scientific Opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single events in the context of its assessments (for oilseed rape GT73, it applies to products with trace levels of GOXv247 protein) remain valid.

For the three-event stack oilseed rape, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analyses of agronomic, phenotypic and compositional characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. An evaluation of environmental impacts and the post-market environmental monitoring (PMEM) plan was also undertaken.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, p. 1–23.

[2] The specific subcombinations are two-event stacks oilseed rape MS8 × GT73 and RF3 × GT73.

[3] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L 157, 8.6.2013, p. 1–48.

The molecular data establish that the events stacked in oilseed rape MS8 × RF3 × GT73 have retained their integrity. Protein expression analyses showed some difference between the levels in the parental lines and the three-event stack which are not unexpected. Therefore, there is no indication of interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

None of the differences identified in seed composition and agronomic and phenotypic characteristics between the three-event stack oilseed rape and its conventional counterpart needs further assessment regarding food and feed safety.

In line with previous assessments, the GMO Panel did not find indications of safety concern of the three-event stack oilseed rape food and feed with trace levels of glyphosate oxidoreductase (GOX) v247 protein (e.g. oil, pollen, toasted meal). However, the GMO Panel is not in a position to assess the safety of the three-event stack oilseed rape MS8 × RF3 × GT73 rapeseed protein isolates or products of this nature, as essential data needed for the safety assessment of GOXv247 are lacking. Rapeseed protein isolates for food is not in the scope of this application. However, products rich in protein, such as rapeseed protein isolates in animal feeding, are covered by the scope of this application and their use is emerging.

In the case of accidental release into the environment of viable seeds of the three-event stack oilseed rape, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape MS8 × RF3 × GT73 plants, or hybridising wild relatives, unless these plants are exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides. However, the GMO Panel is of the opinion that the latter will not result in different environmental impacts compared to conventional oilseed rape. Considering the scope of application EFSA-GMO-NL-2009-75, interactions with the biotic and abiotic environment were not considered to be a relevant issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer of recombinant DNA from the three-event stack oilseed rape to bacteria have not been identified.

Considering the introduced traits, the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concludes that the three-event stack oilseed rape would not raise safety concerns in the case of accidental release of viable GM oilseed rape seeds into the environment, irrespective of possible interactions between the individual events within this three-event stack oilseed rape.

From the three possible subcombinations of oilseed rape MS8 × RF3 × GT73, the scope of this application includes subcombinations that have not been authorised previously (i.e. MS8 × GT73 and RF3 × GT73). The use of oilseed rape MS8 × RF3 is not in the scope of this application. The risk assessment of subcombinations takes as starting point the results of the assessment of single events, data generated for the three-event stack oilseed rape, and all the additional data available on subcombinations. As the risk assessment of the three-event stack oilseed rape could not be completed for products rich in protein, such as rapeseed protein isolates in animal feeding, the GMO Panel is not in a position to complete the safety assessment of subcombinations within the scope of this application.

Given the absence of safety concerns identified on the food and feed derived from the three-event stack oilseed rape MS8 × RF3 × GT73 containing trace levels of the GOXv247 protein, the GMO Panel considers that post-market monitoring of these products is not necessary. However, the GMO Panel is currently not in a position to formulate any recommendation for a potential post-market monitoring for rapeseed protein isolates and products of this nature derived from the three-event stack oilseed rape MS8 × RF3 × GT73.

The GMO Panel considers that the scope of the PMEM plans provided by the applicant is consistent with the scope of the three-event stack oilseed rape and the already assessed two-event stack oilseed rape MS8 × RF3. The GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

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Background

On 20 October 2009, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2009-75, for authorisation of genetically modified (GM) glufosinate-ammonium- and glyphosate-tolerant oilseed rape MS8 × RF3 × GT73 (hereafter referred as three-event stack oilseed rape), submitted by Bayer CropScience AG and Monsanto (hereafter referred as the applicant) within the framework of Regulation (EC) No 1829/2003[1], for food and feed uses, import and processing. Subsequently, the European Commission asked EFSA to consider a modification in the scope of application EFSA-GMO-NL-2009-75 requested by the applicant. The risk assessment presented here is for application EFSA-GMO-NL-2009-75 for the placing on the market of genetically modified glufosinate-ammonium- and glyphosate-tolerant oilseed rape MS8 × RF3 × GT73 and subcombinations that have not been authorised previously (i.e. MS8 × GT73 and RF3 × GT73), independently of their origin, for food and feed uses, import and processing, with the exception of isolated seed protein for food.

After receiving the application EFSA-GMO-NL-2009-75 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application available to the public on the EFSA website.[4] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 29 March 2010, 31 May 2010, 21 September 2010, 18 July 2011, 16 February 2012 and 20 April 2012, EFSA received additional information (requested on 27 November 2009, 16 April 2010, 6 December 2010, 17 August 2011 and 6 March 2012, respectively). On 11 May 2012, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[5] following the requirements of Articles 6(4) and 18 (4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member States had 3 months after the date of receipt of the valid application (until 14 May 2013[6]) to make their opinion known.

The EFSA Panel on Genetically Modified Organisms (GMO) Panel carried out an evaluation of the scientific risk assessment of the three-event stack oilseed rape and subcombinations that have not been authorised previously (i.e. MS8 × GT73 and RF3 × GT73) (referred to as 'subcombinations independently of their origin' according to the Commission Implementing Regulation (EU) No 503/2013[3]). On 2 April 2013, 3 June 2013, 25 June 2013, 6 December 2013, 11 June 2014, 13 February 2015, 17 April 2015, 24 June 2015 and 19 October 2015, the GMO Panel requested additional information from the applicant. The applicant provided the requested information on 16 May 2013, 2 September 2013, 4 February 2014, 12 September 2014, 4 May 2015, 28 May 2015, 13 August 2015 and 10 November 2015. The applicant provided additional information spontaneously on 16 May 2013, 21 January 2016, 25 January 2016 and 11 March 2016.

In giving its Scientific Opinion to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 6 months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this Scientific Opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation, and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

Terms of Reference

In an initial request, the GMO Panel was asked to carry out a scientific risk assessment of oilseed rape 'MS8xRF3xGT73 and all sub-combinations of the individual events independently of their origin (as present in the segregating progeny as well as independent stacks to be placed on the market as such)', for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC)

[4] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2009-00890

[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

[6] The Member States commenting period of application EFSA-GMO-NL-2009-75 was suspended until the clock of the application was re-started following the adoption of the Scientific Opinions of applications EFSA-GMO-BE-2010-81 (authorisation of GM oilseed rape events MS8, RF3, and MS8 × RF3) and EFSA-GMO-NL-2010-87 (authorisation of GM oilseed rape event GT73).

No 1829/2003. Subsequently, the European Commission asked EFSA to consider a modification in the scope of application EFSA-GMO-NL-2009-75 requested by the applicant. The risk assessment presented here is for application EFSA-GMO-NL-2009-75 for the placing on the market of genetically modified glufosinate-ammonium- and glyphosate-tolerant oilseed rape MS8 × RF3 × GT73 and subcombinations that have not been authorised previously (i.e. MS8 × GT73 and RF3 × GT73), independently of their origin, for food and feed uses, import and processing, with the exception of isolated seed protein for food.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

The GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

Assessment

1. Introduction

The application EFSA-GMO-NL-2009-75 covers three events: the three-event stack oilseed rape MS8 × RF3 × GT73 and the two subcombinations that have not been authorised previously (i.e. MS8 × GT73 and RF3 × GT73), independently of their origin (Table 1). The scope of this application is for food and feed uses, import and processing, with the exception of isolated seed protein for food, and excludes cultivation within the European Union (EU). The term 'subcombination' refers to any combination of up to two of the events present in the three-event stack oilseed rape. Subcombinations occur as segregating progeny in harvested seeds of the three-event stack oilseed rape (embryo and albumen), and their safety is part of the assessment of the three-event stack oilseed rape in Section 4 of this EFSA GMO Panel Scientific Opinion.

'Subcombination' also covers combinations of two of the three events MS8, RF3 or GT73 that have either been, or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These are oilseed rape stacks that can be bred, produced and marketed independently of the three-event stack oilseed rape. These stacks, except for the two-event stack oilseed rape MS8 × RF3 that is not in the scope of this application, are risk assessed in Section 5 of this EFSA GMO Panel Scientific Opinion.

The three-event stack oilseed rape was developed to achieve tolerance to the herbicidal active substances glufosinate-ammonium and glyphosate.

Table 1: Oilseed rape events covered by the scope of the application EFSA-GMO-NL-2009-75

Degree of stacking	Events	Unique identifiers
Three-event stack oilseed rape	MS8 × RF3 × GT73	ACS-BNØØ5-8xACS-BNØØ3-6xMON-ØØØ73-7
Two-event stacks oilseed rape	MS8 × GT73	ACS-BNØØ5-8xMON-ØØØ73-7
	RF3 × GT73	ACS-BNØØ3-6xMON-ØØØ73-7

oilseed rape: oilseed rape.

The parental oilseed rape lines MS8 and RF3 have been developed for the production of hybrid seeds MS8 × RF3 resulting in higher yield due to hybrid vigour. The oilseed rape events MS8, RF3 and MS8 × RF3 have been previously assessed all together in respective EFSA GMO Panel Scientific Opinions (see Table 2). No concerns on their safety were identified by the GMO Panel (Table 2).

The safety of oilseed rape GT73 expressing the GOXv247 protein has been previously considered, originally under Regulation (EC) No 258/97 (ACNFP Annual Report, 1995), and subsequently by EFSA GMO Panel in 2004 (EFSA, 2004) and in a renewal opinion in 2009 (EFSA GMO Panel, 2009b). In essence, these assessments were product-driven, recognising that the GOXv247 was absent in the oil used for human consumption and was present only in trace amounts in the toasted meal used in animal feeding. Subsequently, new food products consisting of extracted proteins (protein isolates) from oilseed rape were assessed (EFSA NDA Panel, 2013). As GOXv247 had the potential to be concentrated in such food

materials and consequently human exposure would have been much greater than from the oil, the GMO Panel considered that a more extensive toxicological assessment of the protein was necessary in the context of application EFSA-GMO-NL-2010-87 (EFSA GMO Panel, 2013). To this end, a 28-day toxicity study in rodents was requested. The applicant argued that such a study was not needed to confirm the safety of GOXv247 protein[7] and did not provide the study. Consequently, the GMO Panel concluded that, in the absence of consumption data and repeated dose toxicity studies with the GOXv247 protein, it was not in the position to complete the risk assessment of products of this nature (EFSA GMO Panel, 2013).

Table 2: Single oilseed rape events and two-event stacks oilseed rape already assessed by the GMO Panel

Events	Application	EFSA GMO Panel Scientific Opinions
MS8, RF3 and MS8 × RF3	C/BE/96/01	EFSA (2005)
	EFSA-GMO-RX-MS8-RF3	EFSA GMO Panel (2009a)
	EFSA-GMO-BE-2010-81	EFSA GMO Panel (2012)
GT73	C/NL/98/11	EFSA (2004)
	EFSA-GMO-RX-GT73	EFSA GMO Panel (2009b)
	EFSA-GMO-NL-2010-87	EFSA GMO Panel (2013)

GMO: genetically modified organism.

The EFSA GMO Panel Guidance Documents establish the principle that 'where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability, (b) expression of the events and (c) potential interactions between the events' (EFSA, 2007; EFSA GMO Panel, 2011a).

2. Issues raised by the Member States

Issues raised by the Member States on the three-event stack oilseed rape were considered in this EFSA GMO Panel Scientific Opinion, and are addressed in detail in Annex G of the EFSA Overall Opinion.[4]

3. Updated information on the events

As the publication of the previous EFSA GMO Panel Scientific Opinions on the three single oilseed rape events (EFSA, 2004, 2005; EFSA GMO Panel 2009a,b, 2012, 2013), no safety issue pertaining to the single events has been reported by the applicant.

Bioinformatic analyses on the junction regions for the events, MS8, RF3 and GT73, using the methodology specified in the 2011 EFSA Guidance Document (EFSA GMO Panel, 2011a), confirmed that no known endogenous genes were disrupted by any of the inserts.[8]

Updated bioinformatic analyses of the amino acid sequence of the newly expressed phosphinothricin-acetyl-transferase (PAT), CP4 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and GOXv247 proteins revealed no significant similarities to toxins and allergens.[8] In addition, updated bioinformatics analyses of the newly created open reading frames (ORFs) within the inserts and at their junctions, indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.[8]

In the frame of the present application (EFSA-GMO-NL-2009-75), the GMO Panel requested a 28-day oral toxicity study in rodents with the GOXv247 protein, analytical data on GOXv247 content in specific products, as well as an exposure assessment to this protein. The applicant reiterated the view that a 28-day oral toxicity study was unnecessary to establish the safety of the GOXv247 protein.[9] In addition, the applicant referred to technical difficulties in conducting a 28-day study due to physicochemical properties of the GOXv247 protein.[10]

Based on the above information, the GMO Panel considers that its previous conclusions on the safety of the single events in the context of its assessments (for oilseed rape GT73, it applies to products with trace levels of GOXv247 protein) remain valid. It is noted that the use of protein isolates in animal feeding is emerging. This issue is considered further in Section 4.3.2.

[7] Additional information: 23/4/2012 provided in the context of application EFSA-GMO-NL-2010-87.
[8] Additional information: 12/9/2014 and 4/5/2015.
[9] Additional information: 2/9/2013 and 12/8/2015.
[10] Additional information: 21/1/2016 and 25/1/2016.

4. Risk assessment of the three-event stack oilseed rape MS8 × RF3 × GT73

4.1. Molecular characterisation

Possible interactions that would affect the integrity of the events, the protein expression level, or the biological function conferred by the individual inserts are considered below.

4.1.1. Genetic elements and their biological function

Oilseed rape MS8, RF3 and GT73 are combined by conventional crossing to produce the three-event stack oilseed rape. The structure of the inserts introduced into the three-event stack oilseed rape is described in detail in previous EFSA Scientific Opinions, and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

Table 3: Genetic elements in the expression cassettes of the events stacked in oilseed rape MS8 × RF3 × GT73

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MS8	Pta29 (*Nicotiana tabacum*)[(a)]	–	No	*barnase* (*Bacillus amyloliquefaciens*)	*3′barnase* (*B. amyloliquefaciens*), *nos* (*Agrobacterium tumefaciens*)
	PssuAt (*Arabidopsis thaliana*)	–	No	*bar* (*Streptomyces hygroscopicus*)	3′g7 (*A. tumefaciens*)
RF3	Pta29 (*N. tabacum*)	–	No	*barstar* (*B. amyloliquefaciens*)	*nos* (*A. tumefaciens*)
	PssuAt (*A. thaliana*)	–	No	*bar* (*S. hygroscopicus*)	3′g7 (*A. tumefaciens*)
GT73	35S (*Figwort mosaic virus* (FMV))	–	CTP1 (*A. thaliana*)	*gox247* (*Ochrobactrum anthropi*)	E9 (*Pisum sativum*)
	35S (FMV)	–	CTP2 (*A. thaliana*)	CP4 *epsps* (*Agrobacterium* sp. strain CP4)	E9 (*P. sativum*)

–: when no element was specifically introduced to optimise expression; CTP: chloroplast transit peptide; UTR: untranslated region.
(a): Source of genetic information.

Intended effects of the inserts in oilseed rape MS8 × RF3 × GT73 are summarised in Table 4.

Table 4: Characteristics and intended effects of the events stacked in oilseed rape MS8 × RF3 × GT73

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MS8	Barnase	Based on a gene from *Bacillus amyloliquefaciens*. Barnase is an extracellular ribonuclease secreted by *B. amyloliquefaciens* (Hartley, 1988)	In MS8, the *barnase* coding sequence is under the control of a specific promoter (Pta29). It is only expressed in the tapetum cells during anther development, and results in male sterility
	PAT	Based on a gene from *Streptomyces hygroscopicus.* PAT enzyme acetylates demethylphosphinothricin and phosphinothricin (Thompson et al., 1987)	Expression of PAT in oilseed rape MS8 confers tolerance to glufosinate-ammonium-containing herbicides

Event	Protein	Donor organism and biological function	Intended effects in GM plant
RF3	Barstar	Based on a gene from *B. amyloliquefaciens*. Barstar is the specific intracellular inhibitor of Barnase which protects the bacterial cell from the effects of Barnase (Hartley, 1988)	In RF3, the *barnase* coding sequence is under the control of a specific promoter (Pta29). It is only expressed in the tapetum cells, and leads to restoration of fertility after crossing with the (MS8)
	PAT	Based on a gene from *S. hygroscopicus.* (PAT) enzyme acetylates demethylphosphinothricin and phosphinothricin (Thompson et al., 1987)	Expression of PAT in oilseed rape RF3 confers tolerance to glufosinate-ammonium-containing herbicides
GT73	GOXv247	Based on a gene from *Ochrobactrum anthropi.* The enzyme glyphosate oxidoreductase (GOX) catalyses the conversion of glyphosate to aminophenyl phosphonate and glyoxylate (Barry and Kishore, 1994)	GOXv247 confers tolerance to glyphosate-containing herbicides. GOXv247 expressed in oilseed rape GT73 differs from the wild type GOX at three amino acid positions. These substitutions result in improved kinetic properties of the enzyme
	CP4 EPSPS	Based on a gene from *Agrobacterium* sp. CP4. EPSPS is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995). Glyphosate is a competitive inhibitor of this enzyme	The bacterial CP4 EPSPS confers tolerance to glyphosate-containing herbicides, as it has a greatly reduced affinity towards glyphosate as compared to the plant endogenous enzyme

oilseed rape: oilseed rape; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; GM: genetically modified; PAT: phosphinothricin-acetyl-transferase.

Based on known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the Barnase and Barstar proteins. The Barnase and Barstar proteins are expressed in plant tissues (i.e. tapetum cells of the flower buds only) that are not present in food, or feed derived from the three-event stack oilseed rape.

4.1.2. Integrity of the events in the three-event stack oilseed rape MS8 × RF3 × GT73[11]

The genetic stability of the inserted DNA over multiple generations in the three single oilseed rape events was demonstrated previously (see EFSA GMO Panel Scientific Opinions, Table 2). Southern analyses demonstrated the integrity of the single events in the F_1 generation of the three-event stack oilseed rape.[12]

4.1.3. Information on the expression of the inserts[13]

Plants were grown at three sites (four replicate blocks at each site) under field conditions in Canada in 2011.[14] The presence of the Barnase and Barstar proteins is limited to tapetum cells during anther development. Therefore, an analysis of their levels in other tissues was not considered relevant. The levels of PAT, CP4 EPSPS and GOXv247 proteins in the three-event stack oilseed rape and the three single events were quantified by enzyme-linked immunosorbent assay (ELISA). Protein levels were

[11] Dossier: Part I – Section D5.
[12] Dossier: Part I – Section D2(a).
[13] Dossier: Part I – Section D3.
[14] Additional information: 2/9/2013.

determined in leaves (4–6 leaf and early bolt stages), seed and whole above-ground plant. The plants were treated with glufosinate-ammonium- and/or glyphosate-containing herbicides. Data on seeds (F_2 generation) are reported and discussed below (Table 5).

Table 5: Mean and standard deviations (upper row) and ranges (lower row) of protein levels (μg/g dry weight) in seed from the single oilseed rape events MS8, RF3 and GT73, and oilseed rape MS8 × RF3 × GT73

Protein	Protein levels in seeds			
	MS8 × RF3 × GT73	**MS8**	**RF3**	**GT73**
PAT	0.956 (0.16)	0.632 (0.18)	0.612 (0.15)	NA
	0.698–1.19	0.419–0.885	0.469–0.916	
CP4 EPSPS	11.5 (1.0)	NA	NA	21.1 (1.9)
	9.30–12.9			18.0–24.4
GOXv247	14.0 (2.5)	NA	NA	26.5 (2.2)
	10.3–17.7			22.7–30.3

PAT: phosphinothricin-acetyl-transferase; EPSPS: 5-enoylpyruvyl-shikimate-3-phosphate synthase; GOX: glyphosate oxidoreductase; NA: not assayed.

There are differences between the levels of proteins in seed (F_2 generation) produced by the three-event stack oilseed rape compared to the respective single events. Such differences in expression levels between parental lines and the stack are not unexpected. Data from greenhouse studies[13] suggest that a possible factor explaining the differences in protein expression levels could be the expected difference in zygosity of the transgenes between the parental lines and the three-event stack oilseed rape MS8 × RF3 × GT73.

4.1.4. Conclusion of the molecular characterisation

The molecular data establish that the events stacked in the three-event stack oilseed rape have retained their integrity. Protein expression analyses showed some difference between the levels in the parental lines and the stack which are not unexpected. Therefore, there is no indication of interaction that may affect the integrity of the events and the levels of the newly expressed proteins in the three-event stack oilseed rape.

Based on known biological function of the newly expressed proteins, functional interaction between the Barnase and Barstar proteins are expected. These proteins are expressed in plant tissues (i.e. tapetum cells of the flower buds only) that are not present in food, or feed derived from the three-event stack oilseed rape. No functional interaction is expected for the other newly expressed proteins.

Potential interactions are further assessed for their safety implications to human and animals in Section 4.3, and the environment in Section 4.4.

4.2. Comparative assessment

4.2.1. Choice of comparator and production of material for the comparative assessment[15]

Application EFSA-GMO-NL-2009-75 presents data on agronomic and phenotypic characteristics, as well as on seed composition of the three-event stack oilseed rape derived from field trials performed in Canada in four different growing seasons (Table 6).

The GMO Panel focused on the 2011 field trials for the comparative assessment, because they fulfil all the requirements laid down in the GMO Panel Guidance Document for the risk assessment of food and feed derived from GM plants (EFSA GMO Panel, 2011a). In the 2008 field trials, a GM parental line was used as the comparator. In the 2009 field trials, two negative segregants served as comparators. The GMO Panel is of the opinion that potential unintended differences in the GM plant owing to the genetic modification cannot be discounted using parental lines or negative segregants as the *only* comparators (EFSA GMO Panel, 2011a). Therefore, the GMO Panel considers that the

[15] Dossier: Part I – Section D7.1.

field trials performed in 2008 and 2009 are not appropriate. In the 2010 field trials, a conventional counterpart was used, but natural variation was established using three commercially available GM oilseed rape lines.

Table 6: Overview of comparative assessment studies with the three-event stack oilseed rape MS8 × RF3 × GT73 provided in the application EFSA-GMO-NL-2009-75

Study focus	Study details	Comparators	Commercial reference varieties
Agronomic and phenotypic characteristics; composition	2008, Canada (five locations)[16]	GM parental line MS8 × RF3	GM parental lines MS8 × RF3 (different hybrid than the comparator) and GT73
	2009, Canada (three locations)[16]	Two negative segregants of MS8 × RF3 × GT73	None
	2011, Canada (eight locations)[17]	Conventional counterpart (line A)	Six non-GM varieties
Composition	2010, Canada (four locations)[16]	Conventional counterpart	Three GM varieties

GM: genetically modified; GMO: genetically modified organism; MS8: male sterile line 8.

The 2011 field trials were performed at eight different locations in the typical summer oilseed rape growing regions of Canada.[18] At each site, the following materials were grown in a randomised complete block design with four replicates: oilseed rape MS8 × RF3 × GT73, the conventional counterpart[19] and three different non-GM oilseed rape reference varieties,[20] all treated with required maintenance pesticides and oilseed rape MS8 × RF3 × GT73 treated with the intended herbicides[21] in addition to maintenance pesticides. In these field trials, the comparator was a non-GM oilseed rape line with a genetic background similar to that of oilseed rape MS8 × RF3 × GT73 (as documented by the pedigree) and was therefore considered to be an appropriate conventional counterpart.

The statistical analysis of the agronomic, phenotypic and compositional data from the 2011 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010a, 2011a). This includes a test of difference to determine whether the GM plant is different from its conventional counterpart, and a test of equivalence to determine whether the GM plant falls within the range of natural variation estimated from the non-GM commercial reference varieties. As described by EFSA (EFSA GMO Panel, 2011a), the result of the equivalence test is categorised into four possible outcomes to facilitate drawing conclusions with respect to the presence or absence of equivalence. These four categories are as follows: category I, indicating full equivalence; category II, indicating that equivalence is more likely than non-equivalence; category III, indicating that non-equivalence is more likely than equivalence; and category IV, indicating non-equivalence.

4.2.2. Agronomic and phenotypic analysis[22]

The following nine agronomic and phenotypic endpoints were measured in the in the 2011 field trials (Section 4.2.1): early stand count (establishment), seedling vigour, plant count before and after herbicide applications, days-to-flowering (measured both at the start and end of flowering: flowering start and flowering end), plant height, days-to-maturity and seed yield.

No statistically significant differences were identified between the three-event stack oilseed rape (not treated with the intended herbicides) and its conventional counterpart.

Statistically significant differences between the three-event stack oilseed rape treated with the intended herbicides (in addition to maintenance pesticides) and its conventional counterpart were observed for flowering end, seed yield and plant height. For the endpoints, flowering end and seed

[16] Dossier: Appendices.
[17] Dossier: Appendices; additional information: 13/5/2013.
[18] Six field trials sites were in Saskatchewan (Waldheim, Hague, Radisson, Langham, Maymont and Aberdeen) and two in Alberta (New Sarepta and Tofield).
[19] The conventional counterpart in these field trials was a non-GM oilseed rape line with a genetic background comparable with that of oilseed rape MS8 × RF3 × GT73 (as documented by the pedigree).
[20] In total, six non-GM commercial oilseed rape reference varieties were included in the field trials.
[21] Glyphosate- and glufosinate-ammonium-containing herbicides.
[22] Dossier: Part I – Section D7.4.

yield, the test of equivalence indicated that the estimated means and confidence intervals for the three-event stack oilseed rape were within the equivalence limits from the non-GM oilseed rape reference varieties (equivalence category I; EFSA GMO Panel, 2011a). For plant height, the test of equivalence indicated that the estimated mean lies outside the equivalence limits, and that the confidence interval overlaps with one of the equivalence limits. Hence, non-equivalence for plant height between the three-event stack oilseed rape and the non-GM oilseed rape reference varieties is more likely than equivalence (equivalence category III; EFSA GMO Panel, 2011a). Only the observed difference in plant height is further assessed for its potential environmental impact in Section 4.4.

4.2.3. Compositional analysis[23]

The seeds from the three-event stack oilseed rape harvested from the field trials in Canada in 2011 (Section 4.2.1) were analysed for 81 constituents,[24] including the key constituents recommended by the Organisation for Economic Co-operation and Development (OECD 2011). A total of 21 constituents having more than 50% of the observations below the limit of quantification (LOQ) were excluded from the statistical analysis.[25]

The compositional endpoints that are further considered based on the results of the statistical analysis are presented in Table 7. Statistically significant differences between the three-event stack oilseed rape (not treated with the intended herbicides) and its conventional counterpart were identified for 38 compounds in seeds.[26] The test of equivalence indicated that 36 of these endpoints fell under equivalence category I or II, and seed content of cysteine and methionine fell under equivalence category III (Table 7). Statistically significant differences between the three-event stack oilseed rape (treated with the intended herbicides) and its conventional counterpart were identified for 38 compounds in seeds.[27] The test of equivalence indicated that 35 of these endpoints fell under equivalence category
I or II, and seed content of zinc, cysteine and methionine fell under equivalence category III (Table 7).

After reviewing the biological roles of the compounds in Table 7 and the magnitudes of the changes observed, the GMO Panel did not identify any need for further assessment with regard to food and feed safety.

[23] Additional information: 13/5/2013.

[24] The constituents were: proximates (moisture, crude protein, crude fat, ash, carbohydrates), fibres (neutral detergent fibre, acid detergent fibre), minerals (iron, zinc, copper, manganese, calcium, magnesium, phosphorus, potassium, sodium), vitamins (α-, γ-, δ- and total tocopherols), amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), fatty acids (caproic acid C6:0, caprylic acid C8:0, capric acid C10:0, lauric acid C12:0, myristic acid C14:0, myristoleic acid C14:1, pentadecanoic acid C15:0, pentadecenoic acid C15:1, palmitic acid C16:0, palmitoleic acid C16:1, heptadecanoic acid C17:0, heptadecenoic acid C17:1, stearic acid C18:0, oleic acid C18:1, linoleic acid C18:2, linolenic acid C18:3, gamma-linolenic acid C18:3, arachidic acid C20:0, eicosenoic acid C20:1, eicosadienoic acid C20:2, eicosatrienoic acid C20:3, arachidonic acid C20:4, behenic acid C22:0, erucic acid C22:1, docosadienoic acid C22:2, lignoceric acid C24:0, nervonic acid C24:1), glucosinolates (glucoiberin, progoitrin, epi-progoitrin, glucoraphanin, gluconapoleiferin, glucoalyssin, gluconapin, 4-hydroxyglucobrassicin, glucobrassicanapin, glucobrassicin, gluconasturtiin, 4-methoxyglucobrassicin, neoglucobrassicin, total glucosinolates) and phytic acid.

[25] The constituents were: β-tocopherol, glucoiberin, epi-progoitrin, glucoraphanin, gluconapoleiferin, glucoalyssin, glucobrassicanapin, 4-methoxyglucobrassicin, neoglucobrassicin, caproic acid C6:0, caprylic acid C8:0, capric acid C10:0, lauric acid C12:0, myristoleic acid C14:1, pentadecanoic acid C15:0, pentadecenoic acid C15:1, gamma-linolenic acid C18:3, eicosatrienoic acid C20:3, arachidonic acid C20:4, erucic acid C22:1 and docosadieonic acid C22:2.

[26] The compounds were: crude protein, crude fat, ash, neutral detergent fibre, acid detergent fibre, carbohydrates, manganese, calcium, magnesium, potassium, sodium, δ-tocopherol, alanine, arginine, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, proline, serine, tryptophan, valine, phytic acid, palmitic acid C16:0, palmitoleic acid C16:1, heptadecanoic acid C17:0, stearic acid C18:0, oleic acid C18:1, linolenic acid C18:3, arachidic acid C20:0, eicosadienoic acid C20:2, behenic acid C22:0, lignoceric acid C24:0, nervonic acid C24:1 and gluconapin.

[27] The compounds were: crude protein, crude fat, ash, neutral detergent fibre, acid detergent fibre, carbohydrates, manganese, magnesium, calcium, zinc, δ-tocopherol, alanine, arginine, aspartic acid, cysteine, glycine, glutamic acid, histidine, isoleucine, leucine, methionine, proline, serine, threonine, tryptophan, valine, myristic acid C14:0, palmitic acid C16:0, palmitoleic acid C16:1, heptadecanoic acid C17:0, stearic acid C18:0, oleic acid C18:1, linoleic acid C18:2, linolenic acid C18:3, arachidic acid C20:0, behenic acid C22:0, lignoceric acid C24:0 and nervonic acid C24:1.

Table 7: Compositional endpoints that are further discussed based on results of the statistical analysis: mean (for the conventional counterpart and the three-event stack oilseed rape MS8 × RF3 × GT73) and equivalence limits (from the non-GM oilseed rape reference varieties) estimated from field trials data collected in 2011

Endpoint	Conventional counterpart	Three-event stack oilseed rape MS8 × RF3 × GT73		Equivalence limits from non-GM oilseed rape reference varieties
		Untreated[(a)]	Treated[(b)]	
Zinc (mg/kg dw)	47.6	49.4	50.6*	(36.9, 47.9)
Cysteine (% dw)	0.494	0.487*	0.488*	(0.160, 0.419)
Methionine (% dw)	0.51	0.536*	0.541*	(0.447, 0.531)

dw: dry weight; GM: genetically modified; oilseed rape: oilseed rape.
For the three-event stack oilseed rape, all entries (grey background) fall under equivalence category III; significantly different entries are marked with an asterisk.
(a): Untreated: not sprayed with the intended herbicides.
(b): Treated: sprayed with the intended herbicides.

4.2.4. Conclusion

The GMO Panel concludes that none of the differences identified in seed composition and agronomic and phenotypic characteristics between the three-event stack oilseed rape and its conventional counterpart needs further assessment regarding food and feed safety.

The difference observed in plant height between the three-event stack oilseed rape and its conventional counterpart is further assessed for its potential environmental impact in Section 4.4.

4.3. Food and feed safety assessment

4.3.1. Effect of processing[28]

Based on the outcome of the comparative assessment, processing of the three-event stack oilseed rape into food and feed products is not expected to result in products being different from those of commercial non-GM oilseed rape varieties.

4.3.2. Toxicology

4.3.2.1. Toxicological assessment of newly expressed proteins

The three newly expressed proteins in the three-event stack oilseed rape relevant for the food and feed safety assessment are PAT, CP4 EPSPS and GOXv247 (see Section 4.1.1).

The GMO Panel has previously assessed the safety of these proteins individually in the context of the single events, and no safety concern was identified for the PAT and CP4 EPSPS proteins (see Table 2). Protein expression analyses showed some difference between the levels in the parental lines and the stack which are not unexpected and do not raise concerns (Section 4.1.4).

The safety of the GOXv247 protein expressed in oilseed rape GT73 has been previously considered by the GMO Panel, and is described in Section 1 of this Opinion. In the context of this application, the GMO Panel requested additional information on the GOXv247 protein (see Section 3). However, essential data needed for the safety assessment of the GOXv247 protein were not provided by the applicant.[8] Taken into account all available information on the safety of GOXv247 protein, a weight-of-evidence approach could not be followed to sufficiently reduce current uncertainties mainly due to the lack of a 28-day study. Consequently, the GMO Panel cannot assess the safety of products rich in protein, such as rapeseed protein isolates. Rapeseed protein isolates for food is not in the scope of this application. However, it is noted that the use products rich in protein, such as rapeseed protein isolates in animal feeding, is covered by the scope of this application and their use is emerging (e.g. Nagela et al., 2012; Von Der Haar et al., 2014; http://www.canproingredients.ca/research_development.php).

[28] Dossier: Part I – Section D7.6.

On the basis of the known mode of action of the individual newly expressed proteins, there is no indication for possible interactions relevant for the food and feed safety assessment of the three-event stack oilseed rape.

4.3.2.2. Toxicological assessment of components other than newly expressed proteins

The three-event stack oilseed rape did not show any compositional difference to its conventional counterpart that would require further assessment (see Section 4.2.4). No further food and feed safety assessment of components other than newly expressed proteins is required.

4.3.3. Animal studies with the food/feed derived from GM plants

No animal studies with the three-event stack oilseed rape were provided by the applicant (e.g. 90-day toxicity studies in rodents or feeding studies in young rapidly growing animal species).

No substantial modifications in the composition of the food/feed derived from the three-event stack oilseed rape (see Section 4.2.4), no indication of possible unintended effects and no interactions were identified. Therefore, according to the EFSA Guidance Document (2006a), no animal studies on the food/feed derived from the three-event stack oilseed rape are required.

4.3.4. Allergenicity

For the allergenicity assessment, a weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed proteins, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity (EFSA, 2006a; Codex Alimentarius, 2009; EFSA GMO Panel 2011a). In addition, when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered (EFSA GMO Panel, 2011a). When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed.

4.3.4.1. Assessment of allergenicity of the newly expressed proteins[29]

The GMO Panel has previously evaluated the safety of the PAT, CP4 EPSPS and GOXv247 proteins, and no concerns on allergenicity were identified in the context of the previously assessed applications (e.g. EFSA, 2004, 2005; EFSA GMO Panel 2009a,b, 2012, 2013). No new information on allergenicity of the single events that might change the previous conclusions of the GMO Panel has become available. In addition, there is no information available on the structure or function of the individual newly expressed proteins that would suggest an adverse adjuvant effect of their simultaneous presence in the three-event stack oilseed rape food and feed.

4.3.4.2. Assessment of allergenicity of GM plant products[30]

The GMO Panel regularly reviews the available publications on food allergy to oilseed rape (EFSA GMO Panel, 2014). However, to date, oilseed rape has not been considered to be a common allergenic food[31] (OECD, 2011). Therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM oilseed rape.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 4.1 and 4.2), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the three-event stack oilseed rape compared to that derived from non-GM oilseed.

4.3.5. Nutritional assessment of GM food/feed[32]

The intended trait of the three-event stack oilseed rape MS8 × RF3 × GT73 is herbicide tolerance, with no intention to alter nutritional parameters. Comparison of nutrients and antinutrients of oilseed rape MS8 × RF3 × GT73 with its conventional counterpart and reference varieties did not identify

[29] Dossier: Part I – Section D7.9.1 and additional information 12/9/2014 and 4/5/2015.
[30] Dossier: Part I – Section D7.9.2.
[31] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.
[32] Dossier: Part I – Section D7.10.

differences that would require further safety assessment. From these data, an impact on the nutritional value of food and feed derived from oilseed rape MS8 × RF3 × GT73 is not expected.

4.3.6. Conclusion

In line with previous assessments, the GMO Panel did not find indications of safety concern of the three-event stack oilseed rape food and feed with trace levels of GOXv247 protein (e.g. oil, pollen, toasted meal). However, the GMO Panel is not in a position to assess the safety of the three-event stack oilseed rape MS8 × RF3 × GT73 rapeseed protein isolates or products of this nature, as essential data needed for the safety assessment of GOXv247 are lacking. Rapeseed protein isolates for food is not in the scope of this application. However, products rich in protein, such as rapeseed protein isolates in animal feeding, are covered by the scope of this application and their use is emerging.

4.4. Environmental risk assessment[33]

The three-event stack oilseed rape has been developed for tolerance to glufosinate-ammonium- and glyphosate-containing herbicides, and increased heterosis (hybrid vigour) through the use of the *barnase* gene, which removes male fertility in order to promote hybridisation, and the *barstar* gene which restores male fertility.

The events comprising the three-event stack oilseed rape MS8 × RF3 × GT73 have been previously assessed by the GMO Panel (see Table 2). Therefore, the environmental risk assessment (ERA) of the three-event stack focused on assessing whether the combination of events, or any subcombinations interact to present novel hazards and/or routes to exposure as compared to the single events, the previously assessed two-event stack oilseed rape MS8 × RF3 and conventional oilseed rape, and any new risks arising from these potential interactions.

Considering the scope of the application EFSA-GMO-NL-2009-75 (which excludes cultivation), the ERA of the three-event stack oilseed rape is concerned mainly with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animal fed GM material and bacteria present in environments exposed to faecal material (manure and faeces); and (2) accidental release into the environment of imported viable seeds from the three-event stack oilseed rape during transportation and processing.

4.4.1. Potential unintended effects on plant fitness due to the genetic modification[34]

Oilseed rape (*B. napus* AACC) is an allotetraploid species (*2n* = 38, genome constitution AACC), which has probably evolved through hybridisation and polyploidisation between the two diploid species *B. rapa* (*2n* = 20, AA) and *B. oleracea* (*2n* = 18, CC). It is an annual plant developed for agricultural production.

Survival of oilseed rape outside cultivation areas is possible. Demographic studies and surveys have shown the ability of oilseed rape (*B. napus*) to establish self-perpetuating populations outside agricultural areas, mainly in seminatural and ruderal habitats in different countries (reviewed by Devos et al., 2012; Bauer-Panskus et al., 2013; COGEM, 2013; Hecht et al., 2014; Schulze et al., 2014; Katsuta et al., 2015; Busi and Powles, 2016; Nishizawa et al., 2016). oilseed rape is generally regarded as an opportunistic species, which can take advantage of disturbed sites (e.g. mowed areas) to germinate and capture resources rapidly. In undisturbed natural habitats, oilseed rape lacks the ability to establish stable populations over successive years, possibly due to the absence of competition-free germination sites (Crawley et al., 1993, 2001) and exposure to biological and abiotic stressors likely limiting fitness (COGEM, 2013; Busi and Powles, 2016). Once established in competition-free germination sites, feral populations decline over a period of years (Crawley and Brown, 1995, 2004; Knispel et al., 2008; Squire et al., 2011; Banks, 2014; Busi and Powles, 2016). However, if habitats are disturbed on a regular basis, then feral populations can persist for longer periods (Claessen et al., 2005a,b; Garnier et al., 2006). The persistence or recurrence of a population in one location is variously attributed to replenishment with fresh seed spills, to recruitment from seed emerging from the soil seedbank or shed by resident feral adult plants, or to redistribution of feral seed from one location to another (Pivard et al., 2008a,b).

[33] Dossier: Part I – Section D9.
[34] Dossier: Part I – Section D9.1 and D9.2.

The three-event stack oilseed rape has been developed for tolerance to glufosinate-ammonium- and glyphosate-containing herbicides. The combination of CP4 *epsps*, *gox* and *pat* genes coding for herbicide tolerance traits can provide a potential agronomic and selective advantage to oilseed rape MS8 × RF3 × GT73 plants when exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides.

The applicant presented agronomic and phenotypic data on the three-event stack oilseed rape gathered from field trials conducted in oilseed rape growing areas in Canada during several growing seasons, of which only the 2011 data set allowed a proper agronomic and phenotypic comparison of the three-event stack oilseed rape with its conventional counterpart (see Section 4.2.2). The 2011 data set showed reduced plant height for three-event stack oilseed rape plants, for which non-equivalence between the three-event stack oilseed rape and the non-GM oilseed rape reference varieties is more likely than equivalence, when treated with the intended herbicides. No relevant differences in the other measured plant characteristics were identified (see Section 4.2.2). As fitness is influenced by the plant's performance at various stages of its lifecycle, the observed difference in plant height is unlikely to increase survival, fecundity, competitiveness or invasiveness characteristics of oilseed rape MS8 × RF3 × GT73 plants.

No specific data were provided to compare seed dormancy of the three-event stack oilseed rape plants with its conventional counterpart. However, there is no evidence that tolerance to the herbicidal active substances glufosinate-ammonium or glyphosate would alter seed dormancy of GM herbicide-tolerant oilseed rape plants, compared to their appropriate comparators. Seed dormancy is more likely to be affected by the genetic background of parental genotypes than the acquisition of herbicide tolerance traits.

As the general characteristics of the three-event stack oilseed rape remain unchanged compared to its conventional counterpart, its ability to establish feral populations mostly in ruderal habitats will remain. Seed import spills can therefore lead to the occurrence of feral oilseed rape MS8 × RF3 × GT73 plants, but these are unlikely to establish stable populations over time (reviewed by Devos et al., 2012). Should these plants be exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides, they are likely to exhibit a selective advantage that could increase their occurrence locally (Londo et al., 2010, 2011; Watrud et al., 2011). However, the likelihood of such an event to happen will be restricted to herbicide-treated areas with little biodiversity, so that environmental impacts will be minimal.

Overall, the occurrence of feral oilseed rape MS8 × RF3 × GT73 plants resulting from seed import spills is likely to be low under import conditions, and their occurrence would be confined mostly to ruderal habitats. These plants will therefore not create additional agronomic or environmental impacts compared to their conventional counterparts.

4.4.2. Potential for gene transfer[35]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or through vertical gene flow via the dispersal of pollen from feral plants originating from spilled seeds.

4.4.2.1. Plant-to-bacteria gene transfer

The potential for horizontal gene transfer of the recombinant DNA of the single oilseed events MS8, RF3 and GT73, and the two-event stack oilseed rape MS8 × RF3 was assessed previously by the GMO Panel. No concern as a result of an unlikely, but theoretically possible, horizontal gene transfer of the recombinant genes to bacteria in the gut or other receiving environments was identified (EFSA, 2005; EFSA GMO Panel, 2009a, 2010b, 2012, 2013). Genes of bacterial origin expressed in the three-event stack oilseed rape include the CP4 *epsps* gene of *Agrobacterium* sp., the *gox* gene of *O. anthropi*, the *bar* gene of *S. hygroscopicus*, as well as the *barstar* and *barnase* genes of *B. amyloliquefaciens*, respectively. No pairs of sequences facilitating double homologous recombination were identified. As natural variants of such genes are already present in bacteria occurring in the environment, homologous recombination and gene replacement will not confer novel properties possibly providing a selective advantage to members of the natural microbial communities. Synergistic effects of the recombinant genes in increasing the likelihood for horizontal gene transfer, for instance combinations of recombinogenic sequences, have not been identified. As the three-event stack oilseed rape is produced from conventional crossing, close linkage of the different events is extremely unlikely due to the distances separating them within the plant genome. Therefore, the GMO Panel concludes that, in

[35] Dossier: Part I – Section D9.3.

the context of the scope of the application EFSA-GMO-NL-2009-75, the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this three-event stack oilseed rape to bacteria does not raise any environmental safety concern.

4.4.2.2. Plant-to-plant gene transfer

Considering the scope of the application EFSA-GMO-NL-2009-75 and the biology of oilseed rape, a possible pathway to harm is the potential of occasional feral GM oilseed rape plants originating from seed import spills to transfer recombinant DNA to sexually cross-compatible plants. As pointed out above (Section 4.4.1), the accidental spillage of imported oilseed rape seeds can result in the occurrence of feral plants often in ruderal and disturbed habitats, where they can survive and reproduce.

Oilseed rape is an open pollinating crop plant capable of cross-pollinating with other *Brassica* crops (Eastham and Sweet, 2002). If established adjacent to cross-compatible field crops, then feral oilseed rape MS8 × RF3 × GT73 plants arising from spilled seeds could pollinate oilseed rape crop plants. Shed seed from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops, although the likelihood of this happening is extremely low under an import scenario (Squire et al., 2011; Devos et al., 2012).

Oilseed rape can also spontaneously hybridise with sexually compatible wild relatives. Several oilseed rape × wild relative hybrids have been reported in the scientific literature, but under field conditions transgene introgression has only been confirmed for progeny of oilseed rape × *B. rapa* hybrids (reviewed by Ellstrand et al., 1999, 2013; FitzJohn et al., 2007; Devos et al., 2009). For transgene introgression to occur, feral GM oilseed rape must require some overlap in flowering in time and space with compatible relatives. Subsequently, transgenes must be transmitted through successive backcross generations or selfing, so that they become stabilised into the genome of the recipient (de Jong and Rong, 2013; Garnier et al., 2014). Because of these barriers (Luijten et al., 2015), reported incidences of hybrids and backcrosses with *B. rapa* were therefore found to be low in fields (Jørgensen et al., 2004; Norris et al., 2004; Warwick et al., 2008; Elling et al., 2009), or at ports, along roadsides, and riverbanks (Saji et al., 2005; Aono et al., 2006, 2011; Yoshimura et al., 2006; Elling et al., 2009; Katsuta et al., 2015; Luijten et al., 2015).

The GMO Panel does not consider the occurrence of occasional feral oilseed rape MS8 × RF3 × GT73 plants, pollen dispersal and consequent cross-pollination as environmental harm in itself, as there is no evidence that the herbicide tolerance traits will enhance the vertical gene flow potential, or fitness, persistence or invasiveness of feral oilseed rape MS8 × RF3 × GT73, or cross-compatible plants such as hybridising wild relatives. However, when exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides, occasional cross-compatible plants that acquired the herbicide tolerance traits through vertical gene flow are likely to exhibit a selective advantage, which may lead to their increased occurrence. The likelihood of such an event to happen will be restricted to herbicide-treated areas, so that environmental impacts will be minimal. Therefore, the GMO Panel considers that the acquisition of the herbicide tolerance traits by cross-compatible plants would not create additional agronomic or environmental impacts.

In conclusion, the GMO Panel considers that the likelihood of environmental effects as a consequence of the spread of genes from the three-event stack oilseed rape in Europe will not differ from that of conventional oilseed rape varieties, even after exposure to glufosinate-ammonium- and/or glyphosate-containing herbicides.

4.4.3. Potential interactions of the GM plant with target organisms[36]

Interactions occasional feral oilseed rape MS8 × RF3 × GT73 plants arising from seed import spills with target organisms are not considered to be a relevant issue by the GMO Panel, as there are no target organisms.

4.4.4. Potential interactions of the GM plant with non-target organisms[37]

Considering the scope of the application EFSA-GMO-NL-2009-75, and the low level of exposure to the environment, potential interactions of occasional feral oilseed rape MS8 × RF3 × GT73 plants arising from seed import spills with non-target organisms are not considered to be a relevant issue by the GMO Panel.

[36] Dossier: Part I – Section D9.4.
[37] Dossier: Part I – Section D9.5.

4.4.5. Potential interactions with the abiotic environment and biogeochemical cycles[38]

Considering the scope of the application EFSA-GMO-NL-2009-75, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles are not considered to be a relevant issue by the GMO Panel.

4.4.6. Conclusion

In the case of accidental release into the environment of viable seeds of the three-event stack oilseed rape, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape MS8 × RF3 × GT73 plants, or hybridising wild relatives, unless these plants are exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides. However, the GMO Panel is of the opinion that the latter will not result in different environmental impacts compared to conventional oilseed rape. Considering the scope of the application EFSA-GMO-NL-2009-75, interactions with the biotic and abiotic environment were not considered to be relevant issues. Risks associated with an unlikely but theoretically possible horizontal gene transfer of recombinant DNA from the three-event stack oilseed rape to bacteria have not been identified.

Considering the novel combination of events, the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited exposure levels, the GMO Panel concludes that oilseed rape MS8 × RF3 × GT73 would not raise safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment.

4.5. Conclusion on the three-event stack oilseed rape MS8 × RF3 × GT73

The combination of oilseed rape events MS8, RF3 and GT73 in the three-event stack oilseed rape does not raise issues relating to molecular, agronomic/phenotypic or compositional characteristics requiring further investigations.

In line with previous assessments, the GMO panel did not find indications of safety concern of the three-event stack oilseed rape food and feed with trace levels of GOXv247 protein (e.g. oil, pollen, toasted meal). However, the GMO Panel cannot assess the safety of the three-event stack oilseed rape protein isolates or products of this nature as essential data needed for the safety assessment of the GOXv247 are lacking. Rapeseed protein isolates for food is out of the scope of this application. However, it is noted that products rich in protein, such as rapeseed protein isolates in animal feeding, is covered by the scope of this application and its use is emerging.

Considering the introduced traits and the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concludes that the three-event stack oilseed rape would not raise safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment.

5. Risk assessment of the subcombinations

The risk assessment of subcombinations (see Table 1) takes as starting point the results of the assessment of the single events, data generated for the three-event stack oilseed rape, and all the additional data available on subcombinations. As the risk assessment of the three-event stack oilseed rape could not be completed for products rich in protein, such as rapeseed protein isolates in animal feeding, the GMO Panel is not in a position to complete the safety assessment of subcombinations within the scope of this application.

6. Post-market monitoring

6.1. Post-market monitoring of GM food/feed[39]

Given the absence of safety concerns identified on the food and feed derived from the three-event stack oilseed rape MS8 × RF3 × GT73 containing trace levels of the GOXv247 protein, the GMO Panel considers that post-market monitoring of these products is not necessary. However, the GMO

[38] Dossier: Part I – Section D9.8 and D9.10.
[39] Dossier: Part I – Section D7.11.

Panel is currently not in a position to formulate any recommendation for a potential post-market monitoring for rapeseed protein isolates and products of this nature derived from the three-event stack oilseed rape MS8 × RF3 × GT73.

6.2. Post-market environmental monitoring[40]

The objectives of a post-market environmental monitoring (PMEM) plan according to Annex VII of Directive 2001/18/EC are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the applicant (EFSA, 2006b; EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from the three-event stack oilseed rape and one of its stacks, no case-specific monitoring is required.

The PMEM plans proposed by the applicant for the three-event stack oilseed rape include: (1) the description of an approach involving operators (federations involved in oilseed rape import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system newly established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis.

The GMO Panel considers that the scope of the PMEM plans provided by the applicant is consistent with the scope of the three-event stack oilseed rape and the already assessed two-event stack oilseed rape MS8 × RF3. The GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plans. However, the post-market environmental plan submitted by the applicant for the three-event stack oilseed rape does not include any provision for the two- event stacks not previously assessed by the GMO Panel.

In addition, the GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable seeds of oilseed rape MS8 × RF3 × GT73.

Should risk managers consider the control of feral oilseed rape plants desirable, then the implementation of appropriate communication means for the timely reporting of control failures of feral oilseed rape populations may be recommended.

Overall conclusions and recommendations

The GMO Panel considers that its previous conclusions on the safety of the single oilseed rape events, MS8, RF3 and GT73, in the context of its assessments (for oilseed rape GT73 it applies to products with trace levels of GOXv247 protein) remain valid.

The combination of oilseed rape events MS8, RF3 and GT73 in the three-event stack oilseed rape does not raise issues relating to molecular, agronomic/phenotypic or compositional characteristics requiring further investigations.

In line with previous assessments, the GMO panel did not find indications of safety concern of the three-event stack oilseed rape food and feed with trace levels of GOXv247 protein (e.g. oil, pollen, toasted meal). However, the GMO Panel is not in a position to assess the safety of the three-event stack oilseed rape MS8 × RF3 × GT73 rapeseed protein isolates or products of this nature, as essential data needed for the safety assessment of GOXv247 are lacking. Rapeseed protein isolates for food is not in the scope of this application. However, products rich in protein, such as rapeseed protein isolates in animal feeding, are covered by the scope of this application and its use is emerging.

From the three possible subcombinations of oilseed rape MS8 × RF3 × GT73, the scope of this application includes subcombinations that have not been authorised previously (i.e., MS8 × GT73 and RF3 × GT73). The use of oilseed rape MS8 × RF3 is not in the scope of this application. The risk assessment of subcombinations takes as starting point the results of the assessment of the single events, the data generated for the three-event stack oilseed rape, and all the additional data

[40] Dossier: Part I – Section D9.11.

available on subcombinations. As the risk assessment of the three-event stack oilseed rape could not be completed for products rich in protein, such as rapeseed protein isolates in animal feeding, the GMO Panel is not in a position to complete the safety assessment of subcombinations within the scope of this application.

Considering the introduced traits and the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concludes that the three-event stack oilseed rape would not raise safety concerns in case of accidental release of viable GM oilseed rape seeds into the environment, irrespective of possible interactions between the individual events within this three-event stack oilseed rape. There are no indications of an increased likelihood of spread and establishment of feral oilseed rape MS8 × RF3 × GT73 plants, or hybridising wild relatives, unless these plants are exposed to glufosinate-ammonium- and/or glyphosate-containing herbicides. Moreover, in the light of the scope of the application, data available for various subcombinations, the GMO Panel is of the opinion that any subcombinations of the individual events, including those not previously assessed by EFSA, would raise no environmental safety concerns.

Given the absence of safety concerns identified on the food and feed derived from the three-event stack oilseed rape MS8 × RF3 × GT73 containing trace levels of the GOXv247 protein, the GMO Panel considers that post-market monitoring of these products is not necessary. However, the GMO Panel is currently not in a position to formulate any recommendation for a potential post-market monitoring for rapeseed protein isolates and products of this nature derived from the three-event stack oilseed rape MS8 × RF3 × GT73.

The GMO Panel considers that the scope of the PMEM provided by the applicant is consistent with the scope of the three-event stack oilseed rape and the already assessed two-event stack oilseed rape MS8 × RF3. The GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plans.

Documentation as provided to EFSA

1) Letter from the Competent Authority of the Netherlands, received on 20 October 2009, concerning a request for placing on the market of genetically modified oilseed rape Ms8 × Rf3G73 submitted jointly by Bayer CropScience and Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2009-75).
2) Acknowledgement letter dated 4 November 2009 from EFSA to the Competent Authority of the Netherlands.
3) Letter from EFSA to applicant dated 27 November 2009 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 15 January 2010 providing the timeline for submission of responses.
5) Letter from applicant to EFSA received on 1 February 2010 extending(1) the timeline for submission of responses.
6) Letter from applicant to EFSA received on 2 March 2010 extending(2) the timeline for submission of responses.
7) Letter from applicant to EFSA received on 18 March 2010 extending(3) the timeline for submission of responses.
8) Letter from applicant to EFSA received on 29 March 2010 extending the scope of the application and providing a consolidated version of the dossier.
9) Letter from EFSA to applicant dated 16 April 2010 requesting additional information under completeness check.
10) Letter from applicant to EFSA received on 31 May 2010 providing the timeline for submission of responses.
11) Letter from applicant to EFSA received on 18 June 2010 extending(1) the timeline for submission of responses.
12) Letter from applicant to EFSA received on 2 August 2010 extending(2) the timeline for submission of responses.
13) Letter from applicant (Monsanto) to EFSA received on 16 September 2010 concerning the authorisation to refer to data.
14) Letter from applicant to EFSA received on 21 September 2010 providing additional information under completeness check and replying the request to access data of the application.

15) Letter from EFSA to applicant dated 6 December 2010 requesting additional information under completeness check.
16) Letter from applicant to EFSA received on 24 January 2011 providing the timeline for submission of responses.
17) Letter from applicant to EFSA received on 28 April 2011 extending(1) the timeline for submission of responses.
18) Letter from applicant to EFSA received on 18 July 2011 providing additional information under completeness check.
19) Letter from EFSA to applicant dated 17 August 2011 requesting additional information under completeness check.
20) Letter from applicant to EFSA received on 19 September 2011 providing the timeline for submission of responses.
21) Letter from applicant to EFSA received on 15 December 2011 extending(1) the timeline for submission of responses.
22) Letter from applicant (Monsanto) to EFSA received on 7 February 2012 concerning the authorisation to refer to the application data.
23) Letter from applicant to EFSA received on 16 February 2012 providing additional information under completeness check.
24) Letter from EFSA to applicant dated 6 March 2012 requesting additional information under completeness check.
25) Letter from applicant to EFSA received on 20 April 2012 providing additional information under completeness check.
26) Letter from EFSA to applicant, dated 11 May 2012, delivering the 'Statement of Validity' of application for the placing on the market of genetically modified oilseed rape MS8 × RF3 × GT73 (EFSA-GMO-NL-2009-75), submitted jointly by Bayer CropScience and Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003.
27) Letter from EFSA to applicant dated 11 May 2012 stopping the clock due missing information on single events (application EFSA-GMO-BE-2010-81 and EFSA-GMO-NL-2010-87).
28) Letter from EFSA to applicant dated 25 January 2013 re-starting the clock due to the finalisation of the assessment of applications EFSA-GMO-BE-2010-81 and EFSA-GMO-NL-2010-87.
29) Letter from EFSA to applicant dated 2 April 2013 requesting additional information and stopping the clock.
30) Letter from applicant to EFSA received on 16 May 2013 providing additional information requested and spontaneous supplementary information.
31) Letter from EFSA to applicant dated 3 June 2013 requesting additional information and maintaining the clock stopped.
32) Letter from EFSA to applicant dated 25 June 2013 requesting additional information and maintaining the clock stopped.
33) Letter from applicant to EFSA received on 17 July 2013 providing the timeline for submission of responses.
34) Letter from applicant to EFSA received on 1 August 2013 extending the timeline for submission of responses.
35) Letter from applicant to EFSA received on 2 September 2013 providing additional information.
36) Letter from applicant to EC dated 10 September 2013 requesting a change on the scope of the application.
37) Letter from EFSA to applicant dated 6 December 2013 requesting additional information and maintaining the clock stopped.
38) Letter from applicant to EFSA received on 20 January 2014 providing the timeline for submission of responses.
39) Letter from applicant to EFSA received on 4 February 2014 providing additional information.
40) Letter from EFSA to applicant dated 11 June 2014 requesting additional information and maintaining the clock stopped.
41) Letter from applicant to EFSA received on 25 July 2014 providing the timeline for submission of responses.
42) Letter from applicant to EFSA received on 12 September 2014 providing additional information.
43) Letter from applicant to EFSA received on 9 January 2015 asking clarifications on the progress of the application.

44) Letter from EFSA to applicant dated 10 February 2015 providing clarifications on the progress of the application.
45) Letter from EFSA to applicant dated 13 February 2015 requesting additional information and maintaining the clock stopped.
46) Letter from EFSA to applicant dated 17 April 2015 requesting additional information and maintaining the clock stopped.
47) Letter from applicant to EFSA received on 4 May 2015 providing additional information.
48) Letter from EFSA to applicant dated 17 April 2015 requesting additional information and maintaining the clock stopped.
49) Letter from applicant to EFSA received on 28 May 2015 providing additional information.
50) Letter from applicant to EC dated 11 June 2015 requesting a modification of the scope of the application.
51) Letter from EFSA to applicant dated 24 June 2015 requesting additional information and maintaining the clock stopped.
52) Letter from applicant to EC received on 13 August 2015 providing further clarifications on the scope of the application.
53) Letter from applicant to EFSA received on 13 August 2015 providing additional information.
54) Letter from EFSA to applicant dated 19 October 2015 requesting additional information and maintaining the clock stopped.
55) Letter from applicant to EFSA received on 10 November 2015 providing additional information.
56) Letter from EFSA to applicant dated 19 January 2016 re-starting the clock.
57) Letter from applicant to EFSA received on 21 January 2016 providing additional information spontaneously.
58) Letter from EC to EFSA received on 22 January 2016 regarding the scope of the application.
59) Letter from applicant to EFSA received on 25 January 2016 providing additional information.
60) Letter from EFSA to EC dated 28 January 2016 regarding the scope of the application.
61) E-mail from EFSA to applicant dated 18 February 2016 requesting updated additional information.
62) Letter from applicant to EFSA received on 29 February 2016 regarding clarifications on the scope of the application.
63) Letter from applicant to EFSA received on 11 March 2016 providing updated additional information following the modification of the scope of the application.

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Abbreviations

CTP	chloroplast transit peptide
EC	European Commission
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organism
GOX	glyphosate oxidoreductase
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PMEM	post-market environmental monitoring
UTR	untranslated region